Supporting Information

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SI Materials and Methods

Yeast Strains, Media, and General Methods. The yeast strains used in this work are listed in Table S1 and are congenic to strain JC482 (1). Yeast strains were grown on medium containing 1% yeast extract, 2% peptone, and 2% glucose (YPD) or synthetic complete (SC) medium unless stated otherwise. Strains were sporulated at 24 °C on medium containing 2% Bacto peptone, 1% yeast extract, and 2% potassium acetate. SC medium and media lacking specific amino acids were made as described by Sherman et al. (2). Yeast transformation, manipulation of *Escherichia coli*, and the preparation of bacterial growth media were performed as described previously (3, 4).

For mutagenesis, strain KT1963 (*MAT***a** *leu2 ura3 his3 ipl1-2*) was diluted 1/100 from an overnight culture into H_2O, and 250-μl samples of this dilution were plated on YPD plates. Uncovered plates were irradiated with 50 units of UV light in a UVP-CL1000 UV crosslinker and then incubated for 24 h in the dark at 24 °C before transferring to 33 °C for 3 d. Recovered colonies were mated to KT1829 (*MAT*α *leu2 his3 ipl1-2*) and diploids were tested for dominance of the reversion event. Recessive suppressors were subjected to tetrad analysis. Recessive revertants fell into two classes. In a majority of the cases, two of four spore clones from each tetrad grew at 33 °C, indicating that the reversion event segregated as a single Mendelian mutation. In a few cases, only one in four spore clones was able to grow at 33 °C, suggesting that more than one mutation was responsible for suppression of *ipl1-2*. In most cases, this class of revertant was not studied further. However, in one case, revertant 71, half of the spore clones in each tetrad were cold sensitive (cs), failing to grow at 14 °C. All cs clones grew at 30 °C but only half were able to grow at 33 °C. These results indicate that two mutations in revertant 71 were necessary for growth at 33 °C and that one of these had an accompanying cs phenotype. Sequence analysis of the *TCO89* ORF from revertant 71 revealed 11 mutations (T5G, A12T, A53G, A93C, G94A, T95A, T1934A, A2293T, T2296A, T2297C, and C2299A).

To assay for chromosome III loss in diploids, *ipl1-2* homozygous and heterozygous strains were grown to log phase at 24 °C in YPD before shifting to 30 °C. At 2-h intervals, aliquots of the cultures were serially diluted and plated on YPD plates to determine cell viability. Another aliquot was mixed with a 10-fold excess of a culture of *MAT***a** yeast strain KT82 (*MAT***a** *hom3*), collected onto a nitrocellulose filter, and incubated on YPD medium at 24 °C. After 12 h, cells were transferred from the filters to H_2O and plated on synthetic medium to select for growth of the mated cells.

The *tco89::kanMX* and *tor1::kanMX* deletion strains from the deletion collection (5) obtained from Open Biosystems were amplified by PCR using flanking primers (5′ CCATCTAGACTATCAATGATGGACGAC and 5′ CTTGAGCTCTAGTGATTCTGACCGATAG for *tco89* and 5′ CATTACTAACTGCTGAGG and 5′ CGTTTTGGTGATGAAGATG for *tor1*) and iProof high-fidelity DNA polymerase (Bio-Rad), according to the manufacturer's instructions. The amplified cassettes were used to transform the KT1112 × KT1113 wild-type diploid strain, drug-resistant prototrophic transformants were sporulated, and haploid meiotic segregants were isolated by tetrad analysis. All deletions, truncations, and integrations were confirmed by genomic PCR using primers in flanking sequences.

Immunoblot Analysis. To assess tagged protein levels, total protein was prepared from cultures by lysis in tricholoracetic acid (6, 7), and equal volumes were applied to 4–20% gradient gels (Bio-Rad). Gels were blotted to nitrocellulose (Bio-Rad), and immunoblot analysis was performed as described (7) using anti-Myc 9E10 ascites antibody or BD Living Colors A.v. monoclonal anti-GFP (JL-8) antibody with HRP-conjugated secondary antibody and subsequent detection using ECL reagents (Pierce). Protein levels were quantitated from immunoblots using the Chemidoc system with Quantity One software V 4.6.6 (Bio-Rad), with phosphoglycerate kinase (Pgk1) as a loading control (anti-Pgk) (Molecular Probes).

For the Dam1 Ser257 analysis, yeast cells were lysed and prepared for SDS/PAGE by sequential treatment with alkali and trichloroacetic acid, as previously described (8). The Ser257-*P* antibody was used at a concentration of 0.5 μg/mL after pretreatment for 30 min with 5.0 μg/mL of nonphosphorylated peptide, and anti-Dam1 antibody was used at 1:5,000 final concentration (9). The phospho-specific signal was quantified as previously described (9).

For histone H3 Ser10 analysis, overnight cultures grown in YPD at 24 °C were diluted to ~1×10^7 cells/mL and grown for 2 h at 24 °C. Cultures were then split and grown for 2 h more, one-half of each culture at 24 °C and the other half at 30 °C. Cultures were placed on ice, and 1.6-mL samples were pelleted and resuspended in 1× sample buffer with phosphatase inhibitors. Glass beads were added to 1/2 sample volume, and cells were lysed 5 min at 4 °C. After 5 min at 99 °C, samples were centrifuged briefly, and 30 μl of supernatant was applied to precast 4–20% gradient gels (Bio-Rad). Gels were blotted to PVDF membrane as recommended by the manufacturer, and blots were blocked with 5% milk in tris buffered saline with 0.1% tween-20 and probed with anti-phospho-histone H3 (serine 10) (Millipore) and HRP-conjugated goat anti-rabbit IgG (Bio-Rad). ECL reagents (Pierce) were used to develop the blots, and signal was detected and quantitated using the Chemidoc system. Blots were then stripped using Western ReProbe (G-Biosciences) at 1× for 4–5 h, reblocked, and reprobed using antihistone H3 (Millipore) to obtain total histone H3 signal.

Microscopy. For live-cell imaging, cells were placed onto a pad of 2% agarose in SC medium and imaged through an Olympus UPlanFl 100×/1.3 NA objective using a CoolSNAP HQ charge-coupled device camera. For imaging mCitrine and GFP, a 41001 filter set was used (Chroma Technology). For imaging mCherry, a TRITC filter set (Olympus) was used. Slidebook, version 4 software (Intelligent Imaging Innovations), was used to control camera acquisition and the *z* axis stepping motor (Ludl Electronic Products). For the image analysis of Glc7-mCitrine fluorescence in Fig. 5 *A* and *B*, wild-type cells expressing Glc7-mCitrine were mixed with *tco89-17*, *YPI1-GFP*, or *tco89-71 YPI1-GFP* mutant cells expressing Glc7-mCitrine and Pom34-mCherry and imaged together in the same field. The mCherry fluorescence signal was used to distinguish the wild type from mutant cells. Fluorescence images (binned 2 × 2) were acquired in a series of *z*-axis planes (0.5 μm apart). Images in five planes were converted into a *z*-axis projection, and the average fluorescence in nine adjacent pixels was measured in the nucleus and cytoplasm of each cell using ImageJ 1.43. software (10). Care was taken to avoid the high fluorescent signal that corresponds to the nucleolus and low fluorescence in the cytoplasm associated with the vacuole. For the quantitation of fluorescence in Fig. 4*D*, wild-type cells expressing Glc7-mCitrine and Pom34-mCherry (KT3242) were grown to log phase in YPD medium and then

incubated in YPD medium containing 10 mM caffeine or 10 mM rapamycin. After a 2-h incubation at 30 °C, cells were collect by centrifugation, washed in YPD, and mixed with congenic untreated cells (KT3249) that lacked the Pom34-mCherry fusion protein. The mixture of treated and untreated cells were imaged in the same field as described above.

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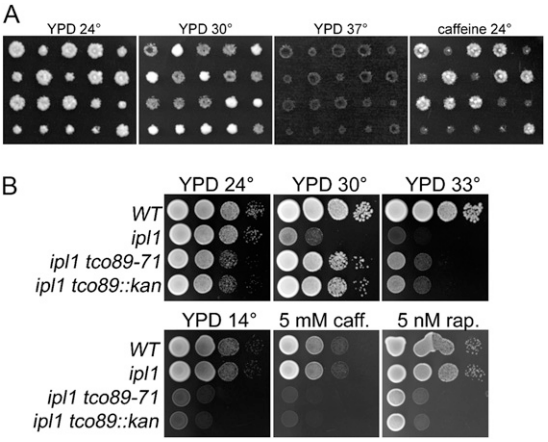

Fig. S1. (*A*) Cosegregation of *ipl1-2* suppression and caffeine sensitivity in a *tco89-71* mutant. A diploid strain homozygous for *ipl1-2* and heterozygous *tco89-71* (KT1829 × KT3002*)* was subjected to tetrad analysis. The four spores from each tetrad were separated into vertical rows on YPD medium and allowed to grow at 24 °C for 3 d. The spore clones were then replicate-plated to YPD medium at 24 °C, 30 °C, and 37 °C and to YPD containing 2.5 mM caffeine at 24 °C and imaged after 48 h. Note the cosegregation of *ipl1* suppression and caffeine sensitivity. (*B*) *toc89-71* and *tco89::kanMX* mutants have identical phenotypes. Cultures of *WT* (KT1112), *ipl1-2* (KT1963), *ipl1-2 toc89-71* (KT3002), and *ipl1-2 tco89::kanMX* (KT3098) strains were serially diluted onto YPD medium and YPD containing 5 mM caffeine or 5 nM rapamycin and incubated at the designated temperature before imaging. The caffeine and rapamycin panels were incubated at 24 °C. Plates were incubated for 40 h, with the exception of the 14 °C panel, which was incubated for 13 d.

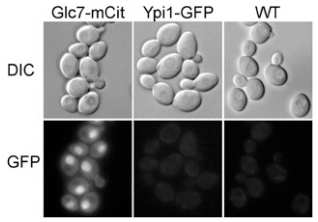

Fig. S2. Ypi1-GFP fluorescence is at or below the limit of detection. *GLC7-mCitrine* (KT3242), *YPI1-GFP* (KT3248), and WT (KT3251) cells were grown to midlog phase in YPD medium at 30 °C and imaged using the GFP filter cube that was used for the image analysis presented in Fig. 4. The same normalization values were used for each fluorescence image.

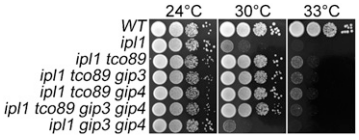

Fig. S3. *gip3Δ* and *gip4Δ* mutations do not influence the temperature sensitivity of *ipl1-2 tco89-71* mutants. Cultures of *WT* (KT1113), *ipl1-2* (KT1829), *ipl1-2 toc89-71* (KT3003), *ipl1-2 toc89-71 gip3Δ::kanMX* (KT3090), *ipl1-2 toc89-71 gip4Δ::kanMX* (KT3094), *ipl1-2 toc89-71 gip3Δ::kanMX gip4Δ::kanMX* (KT3119), and *ipl1-2 gip3Δ::kanMX gip4Δ::kanMX* (KT3117) strains were serially diluted onto YPD medium and incubated at the designated temperatures for 48 h before imaging.

Table S1. Genetic linkage of *tco89-71* with *IPL1* and *PPQ1*

Cross	Markers	P:NP:T	Map distance (cM)
EG1885	*ipl1-2–tco89-71*	32:0:15	16
EG1891	*ipl1-2–tco89-71*	28:0:16	18.2
EG1891	*ppq1::URA3-tco89-71*	44:0:0	<1.1

P:NP:T, parental ditype: nonparental ditype: tetratype.

Table S2. Strain list

Name	Description	Source
KT1112	*MAT***a** *leu2 his3 ura3-52*	(1)
KT1113	*MATα leu2 his3 ura3-52*	(2)
KT1774	*MAT***a** *glc7-129*	(3)
KT1829	*MATα ipl1-2*	(3)
KT1963	*MAT***a** *ipl1-2*	(4)
KT1964	*MAT***a** *glc7-109 ipl1-2*	(3)
KT1965	*MAT***a** *glc7-127 ipl1-2*	(3)
KT1966	*MAT***a** *glc7-109*	(3)
KT1967	*MAT***a** *glc7-127*	(3)
KT1968	*MATα glc7-127 ipl1-2*	(3)
KT1969	*MATα glc7-127*	(3)
KT2088	*MATα his3::GFP-lacI::HIS3 trp1::lacO$_{256}$::TRP1*	This study
KT2941	*MATα tco89-71 ipl1-2*	This study
KT2958	*MAT***a** *tco89-71*	This study
KT2959	*MATα tco89-71*	This study
KT2998	*MATα YPI1-GFP::kanMX6 tco89-71*	This study
KT3000	*MATα YPI1-GFP::kanMX6 tco89-71 ipl1-2*	This study
KT3002	*MAT***a** *tco89-71 ipl1-2*	This study
KT3003	*MATα tco89-71 ipl1-2*	This study
KT3004	*MATα tco89-71 glc8::URA3 ipl1-2*	This study
KT3005	*MATα glc8::URA3 ipl1-2*	This study
KT3007	*MATα YPI1-GFP::kanMX ipl1-2*	This study
KT3010	*MATα tor1Δ::HIS3 ipl1-2*	This study
KT3011	*MATα tor1Δ::HIS3 YPI1-GFP::kanMX6 ipl1-2*	This study
KT3030	*MATα his3::GFP-lacI::HIS3 trp1::LacO$_{256}$::TRP1 tco89-71 ipl1-2*	This study
KT3031	*MATα his3::GFP-lacI::HIS3 trp1::LacO$_{256}$::TRP1 ipl1-2*	This study
KT3076	*MAT***a** *glc7-129 ipl1-2*	This study
KT3090	*MATα tco89-71 ipl1-2 gip3Δ::kanMX*	This study
KT3094	*MATα tco89-71 ipl1-2 gip4Δ::kanMX*	This study
KT3117	*MATα ipl1-2 gip3Δ::kanMX gip4Δ::kanMX*	This study
KT3119	*MATα tco89-71 ipl1-2 gip3Δ::kanMX gip4Δ::kanMX*	This study
KT3242	*MATα GLC7-mCitrine::SpHis5 POM34-mCherry::SpHis5*	This study
KT3245	*MATα GLC7-mCitrine::SpHis5 POM34-mCherry::SpHis5 tco90-71*	This study
KT3247	*MATα GLC7-mCitrine::SpHis5 POM34-mCherry::SpHis5 YPI1-GFP::kanMX*	This study
KT3248	*MATα POM34-mCherry::SpHis5 YPI1-GFP::kanMX*	This study
KT3250	*MATα GLC7-mCitrine::SpHis5 POM34-mCherry::SpHis5 YPI1-GFP::kanMX tco89-71*	This study
KT3251	*MATα POM34-mCherry::SpHis5*	This study
KT3252	*MATα GLC7-mCitrine::SpHis5*	This study

All strains are congenic to KT1113 and KT1112 and have the *leu2 his3 ura3-52* mutations.

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